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THE EFFECTS OF ACUTE ALTERATIONS IN HEMODYNAMICS OXYGEN AVAILABILITY AND ACID-BASE BALANCE ON THE PERMEABILITY OF THE GASTRIC MUCOSA

Annual and Final Report

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Using a previously described model for acute gastric mucosal ulcerogensis, developed under the auspices of the current contract (Gastroent. 68: 699, 1975), studies for this laboratory during the period covered by this annual progress report indicate the following:

(1) Systemic 16,16 DM PGE_2 is cytoprotective in bile acid treated gastric mucosa. Such cytoprotection is dose dependent, is not a function of mucosal blood flow, and is unrelated to bicarbonate secretion. Rather the data suggest that maintenance of normal mucosal transport is responsible.

(2) Deconjugated bile acids induce significantly greater physiologic damage to proximal gastric mucosa then do conjugated bile acid, a circumstance which may be related to the greater capacity of deconjugated bile acids to extract integral proteins from the lipid layer of cell membranes.

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A

20. Abstract. (Con't)

(3) Surface epitheleal cells probably represent the site responsible for the restrictive permeability to cations characteristic of normal proximal gastric mucosa. (AW)

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PROGRESS REPORT: CONTRACT DAMD 17-74-C-4014

I. TITLE OF RESEARCH CONTRACT:

The Effects of Acute Alterations in Hemodynamics, Oxygen Availability, and Acid-Base Balance on the Permeability of the Gastric Mucosa.

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IV. PROGRESS REPORT:

(1) Cytoprotection by systemic prostaglandins in bile acid treated gastric mucosa: dose dependent and unrelated to secretion of HCO_3^-. Both topical and systemic prostaglandins (PG) are cytoprotective in proximal gastric mucosa. Postulated mechanisms include: (1) maintenance of normal mucosal transport, (2) increased mucosal blood flow (MBF), and (3) active secretion of HCO_3^- which yields: $HCO_3^- + H^+ \xrightarrow{C.A.} H_2O + CO_2$. If (3) is true, luminal solutions should demonstrate: (1) ↑ H+ loss, (3) ↓ osmolarity, and (4) ↑ Na+ gain, assuming coupled Na+ and HCO_3^- flux. Using ex-vivo chambered mucosa, these possibilities were tested in 4 groups of dogs during 9 sequential 15 min. study periods. Mucosae of group A (n=8) were subjected during 1-3 to topical acid test solution alone (ATS); during 4-6, to ATS containing 2 mM taurocholic acid (TC); and during 7-9, to ATS+TC+Vasopressin (VP=$5x10^{-3}$ U/Kg-min., IA). Groups B,C, and D (n=4-6) differed only in that 16,16 DM PGE_2 was continuously infused IV at $1x10^{-2}$, $2x10^{-2}$, and $4x10^{-2}$ ugm/Kg-min., respectively. Parameters evaluated included: (1) net volume (V=ml), ion (μEq), and osmolar (μOsm) fluxes, (2) electrical potential difference (PD), (3) MBF (ml) determined using radiolabeled microspheres, and (4) severity of mucosal damage, the lesion index (LI), graded 0-5 by an independent observer. Results: In mucosae exposed to ATS alone, no differences were apparent between groups. Control mucosae exposed to ATS+TC demonstrated ↑H+ loss, ↑Na+ gain, ↑MBF, ↑osomolar loss, and ↓PD. PG significantly reversed these changes toward normal but only at $2x10^{-2}$ ugm/Kg-min. Results/15 min. in ischemic mucosa:

	ΔH+	ΔNa+	ΔK+	ΔV	ΔOsm	MBF	LI
Control	-247±27	+230±16	+19±2	+1.3±0.1	-76±36	2.7±0.2	3.3±0.3
DM PGE_2 $2x10^{-2}$	-113±26*	+139±15*	+9±2*	+0.7±0.1*	+30±50*	2.5±0.6	1.8±0.4*

*P< 0.05 vs Control

All other doses of PG were not different from control. Thus, (1) systemic 16,16 DM PGE_2 is cytoprotective in bile acid treated gastric mucosa; (2) cytoprotection is dose dependent; (3) it is not a function of MBF; and (4) since none of the criteria outlined are filled, it is unrelated to HCO_3^- secretion. Rather, the data suggest that maintenance of normal mucosal transport is responsible.

(2) Deconjugated bile acids are more damaging to gastric mucosa than are conjugated bile acids. A recent clinical report suggests that greater than normal amounts of deconjugated bile acids (BA) appear in the gastric content of post-operative patients who develop stress ulcer disease. However, the pathophysiologic significance of this finding is unclear; although the damaging properties of deconjugated BA on distal ileal and colonic mucosa have been convincingly demonstrated, the intervening small bowel is inconsistently effected. The present study was designed to examine the capacity of the primary BA, cholic, in both its taurine-conjugated (TC) and the deconjugated (C) forms, to induce "back-diffusion" of H+, to decrease transmural electrical potential difference, and to increase gastric mucosal blood flow, all convincing indices of physiologic mucosal injury.

Healthy adult mongrel dogs were used. Under general endotracheal anesthesia (pentobarbital, 30mg/Kg IV), each was prepared with an ex-vivo chambered wedge of proximal gastric wall which was then studied during 9 sequential 15 minute periods. The mucosae of control group A (n=5) was subjected to topical neutral test solution (NTS=160mM NaCl, 4gm PEG, 5μCi ^{14}C-PEG, buffered to pH 7.4) during periods 1-6 and to topical acid test solution (ATS=100mM HCl, 60mM NaCl, 4gm PEG, 5uCi ^{14}C-PEG, pH 1.2) during periods 7-9. Group B (n=5) was exposed to NTS during 1-3, to NTS containing 5mM TC during 4-6, and to ATS alone during 7-9. Group C (n=5) was treated similarly except that the BA employed was 5mM C. Parameters evaluated during each period included the transmural electrical potential difference (PD, mV), net H+ flux (ΔH+, μEq) and mucosal blood flow (MBF, ml/min.), determined during periods 1, 4, and 6 using radiolabeled microsphere embolization.

Bile acids in neutral test solution produced a significant decrease in PD (A=-62 ± 1 mV, B=-47 ± 2 mV, C=-47 ± 2 mV, $p<0.01$) but no change in ΔH+ (nonexistant in any group) or MBF (A=-35 ± 15%, B=-18 ± 11%, C=-32 ± 3%), compared to neutral test solution alone. The results upon subsequent mucosal exposure to topical acid test solution alone (periods 7-9) are summarized below:

	PD (mV)	ΔH+ (μEq / 15 min)	MFB (%Δ vs per. 1)
Group A (ATS alone)	-62 ± 1	-53 ± 19	-17 ± 16
Group B (ATS $\bar{p}$ TC)	-54 ± 1^{x}	-57 ± 14	-23 ± 24
Group C (ATS $\bar{p}$ C)	$-38\pm2^{x+}$	$-226\pm21^{x+}$	$+143+40^{x+}$

x = $p < 0.05$ vs A; + = $p < 0.05$ vs B

The data indicate that, compared to TC, mucosae exposed to acid following topical C demonstrate a significantly greater decrease in

PD and increase in both $\Delta H+$ and MBF.

Thus, at identical and physiologic concentrations, the deconjugated form of the primary BA, cholic, induces significantly greater physiologic damage to proximal canine gastric mucosa than does its taurine conjugate. This circumstance may be related to the greater capacity of deconjugated BAs to extract integral proteins from the lipid layer of cell membranes.

(3) Influence of Topical Bile Acids on Gastric Mucosal Surface Epithelial Cells (SECs). Considerable effort has been devoted toward elucidating the influence of topical bile acid application to proximal gastric mucosa at both high (7.0) and low (1.2) pH on the morphology of the surface epithelial cells, in an attempt to assess the hypothesis that SECs are the anatomic site of the so called "gastric mucosal barrier".

The study design involved two sets of experiments. The first assessed alterations in net ion fluxes, electrical potential difference, and mucosal blood flow during graded (and minimal) injury to the "barrier" and is described under Item (2) above. An additional group of animals (group D, n=5) was included. The mucosae of this group were treated in an manner analogous to group C above except that the concentration of cholic acid employed was 2.5 mM. Compared to NTS alone, NTS + 2.5 C resulted in a significant decrease in potential difference (to -55+4 mV) but no change in Δ H+ or MBF. The results in this group during periods 7-9 (ATS following NTS + 2.5 mm C);

	PD (mV)	ΔH+ (μEq/15 min.)	MBF (%Δ vs. per. 1)
Group D	-50 ± 6	-149 ± 24	$+68 \pm 25$

Thus the extent to which the physiologic parameters in mucosal function were deranged was a function of the concentration of cholic acid in contact with the mucosa.

In a second set of studies, correlation of the physiologic alterations observed in the first with alterations in SEC morphology was sought. This was accomplished by subjecting groups of animals to the same treatment as outlined above; i.e., group A (n=4) to NTS during 1-6, ATS during 7-9; group B (n=4) to NTS during 1-3, NTS + 5 TC during 4-6, ATS during 7-9; group C (n=5) to NTS during 1-3, NTS + 5 C during 4-6, ATS during 7-9; and group D (n=3) to NTS during 1-3, NTS + 2.5 C during 4-6, ATS during 7-9. The physiologic parameters outlined above were not reassessed; rather, biopsies of the mucosa were obtained during the first five minutes of periods 3, 6, 7, and 9 in each animal. Following fixation, coating, and mounting, each specimen was examined using scanning electron microscopy (SEM). The source of the specimen was unknown to the viewer who estimated the percentage of the entire surface area of the scanned specimen involved with the processes described below.

Three distinct modes of SEC response were identified. The first, exocytosis, involved the extrusion of single, non-viable, SECs into the lumen, always from the intrafoveolar portion of the mucus membrane. Approximately two such events could be identified in the entire surface area scanned at 2400 X (fig. 1). This was the only process identified in the mucosa exposed to NTS alone or to NTS containing bile acids. Despite the observed differences in PD and net sodium flux under the latter circumstance, no qualitative differences were observed in the numbers of exocytotic cells at any given magnification between bile acid species or concentrations (figs. 2, 3). Once exocytosis was accomplished, the adjacent SECs appeared to form an encroaching rosette, seeming to enfold themselves on the space vacated by the shed cell (fig. 4). Exocytosis is apparently a normal mechanism by which ageing SECs are eliminated from the gastric mucosa.

The second process identified was apical mucus expulsion, in which individual SECs or groups of SECs shed the mucus granules present in the apical portion of the cell (fig. 5), leaving a pitted appearance to the surface epithelium. The cells remained attached at their baso-lateral membranes and appeared viable (fig. 6). The numbers of cells involved varied from a few (fig. 7) to more then 50% of the total cellular complement scanned at 3300 X (fig. 8) within the same specimen. As with exocytosis, this process was always located at the apices of the ridges between gastric pits. Apical mucus expulsion was the only process identified in specimens exposed to acid following exposure either to NTS along (group A) or to NTS + 5 mM TC (group D). It is important to note that no significant differences in the physiologic status of the barrier were apparent between these groups, and that, concomitantly, no qualitative differences were apparent in the surface epithelium.

The third process, SEC desquamation, was seen only in mucosa exposed to ATS after NTS containing cholic acid, and was associated with all of the physiologic parameters associated with damage to the barrier. With 5 mM C (group C), entire sheets of SECs were completely exfoliated, revealing a totally denuded underlying lamina propera. This process was apparent in all of the specimens examined and was invariably located in the intrafoveolar portion of the mucus membrane. Normal SECs could be identified in the gastric pits, however, (figs. 9, 10). In one-third of the specimens treated with this concentration of cholic acid, this process was so extensive ~~that~~, in approximately 10% of the surface area scanned, the lamina propera was completely exposed around the entire foveolar circumference. In these areas, desquamation extended into the pits themselves for short distances (fig. 11). Despite this electron micrographic appearance, however, gross mucosal damage was not apparent.

SEC desquamation was also noted in all speciments to which ATS was applied following prior treatment with 2.5 mM cholic acid. However, as a comparison of fig. 9 with fig. 12 and fig. 13 with fig. 14 indicates, the extent of the damage was considerably less: fewer intrafoveolar cells were non-viable, the lamina propria was not denuded, a smaller surface area was involved (approximately 30% of the area scanned), and desquamation extending into the gastric pits was never encountered. Concomitantly, the physiologic parameters associated with barrier disruption were deranged to a much lesser extent (see above).

Based on these data, the following conclusions are drawn: 1.) Exocytosis of individual SECs is a normal event which probably represents the manner in which the mucosa rids itself of aging surface cells. It is the only process identified in mucosa exposed to neutral solution (pH 7).

2.) Mucosa exposed to topical cholic acid at neutral pH demonstrates an impaired ability to maintain an electrical gradient as well as a modest luminal gain of sodium, a reflection, no doubt, of impaired active transport. This is unassociated with significant morphologic alterations in SECs relative to that observed with neutral test solution alone. However, it is likely that preservation of these active transport processes is a critical factor in maintaining mucosal integrity because the subsequent application of acid alone produces all of the physiologic alterations associated with "barrier" damage.

3.) The principle response of non-pretreated mucosa exposed to acid solution (pH 2) is apical mucus expulsion. SECs remain entirely viable under these circumstances and the physiologic "barrier" remains intact.

4.) In mucosa which has been pretreated with physiologic concentrations of cholic acid at neutral pH, however, the topical application of acid rapidly results in all the physiologic changes characteristic of "barrier disruption". This is associated with desquamation of entire sheets of SECs, always from the intrafoveolar ridges. Like the physiologic parameters assessed, this process is dose dependent. These data suggest (although they do not prove) that the SECs themselves represent the site responsible for the restrictive permeability to cations characteristic of normal gastric mucosa.

V. PUBLICATIONS DURING THE CONTRACT YEAR:

1. Ritchie, W.P., Jr.:Pathophysiology of Erosive Gastritis and Stress Ulceration. Gastrointestinal Hemorrhage, Fiddian-Green, R., Turcotte, J.G., Eds.; pg. 155-166, New York, Grune and Stratton, 1980.

2. Cloud, W.G., and Ritchie, W.P., Jr.: Deconjugated Bile Acids are More Damaging to Gastric Mucosa than are Conjugated Bile Acids. Surg. Forum 31:115-117, 1980.

3. Ritchie, W.P., Jr., Felger, T.S.: Differing Ulcerogenic Potential of Dihydroxy and Trihydroxy Bile Acids in Canine Gastric Mucosa. Surgery, 89:342-347, 1981.

4. Ritchie, W.P., Jr.: Bile Acid-Induced Acute Gastric Mucosal Damage: A Useful Experimental Model. Scand. J. Gastroent. In press.

5. Ritchie, W.P., Jr.: Acute Gastric Mucosal Injury Induced by Topical Bile Acids. Basic Mechanisms of Gastrointestinal Mucosal Injury. John Harmon, Jr., Ed.; Baltimore, Williams and Wilkins. In press.

6. Ritchie, W.P., Jr.: Role of Bile Acid Reflux in Acute Hemorrhagic Gastritis. World J. Surg. In press.

7. Ritchie. W.P., Jr., Felger, T.S.: Mediators of Bile Acid Induced Alterations in Gastric Mucosal Blood Flow (MF) Gastroenterology. In press.

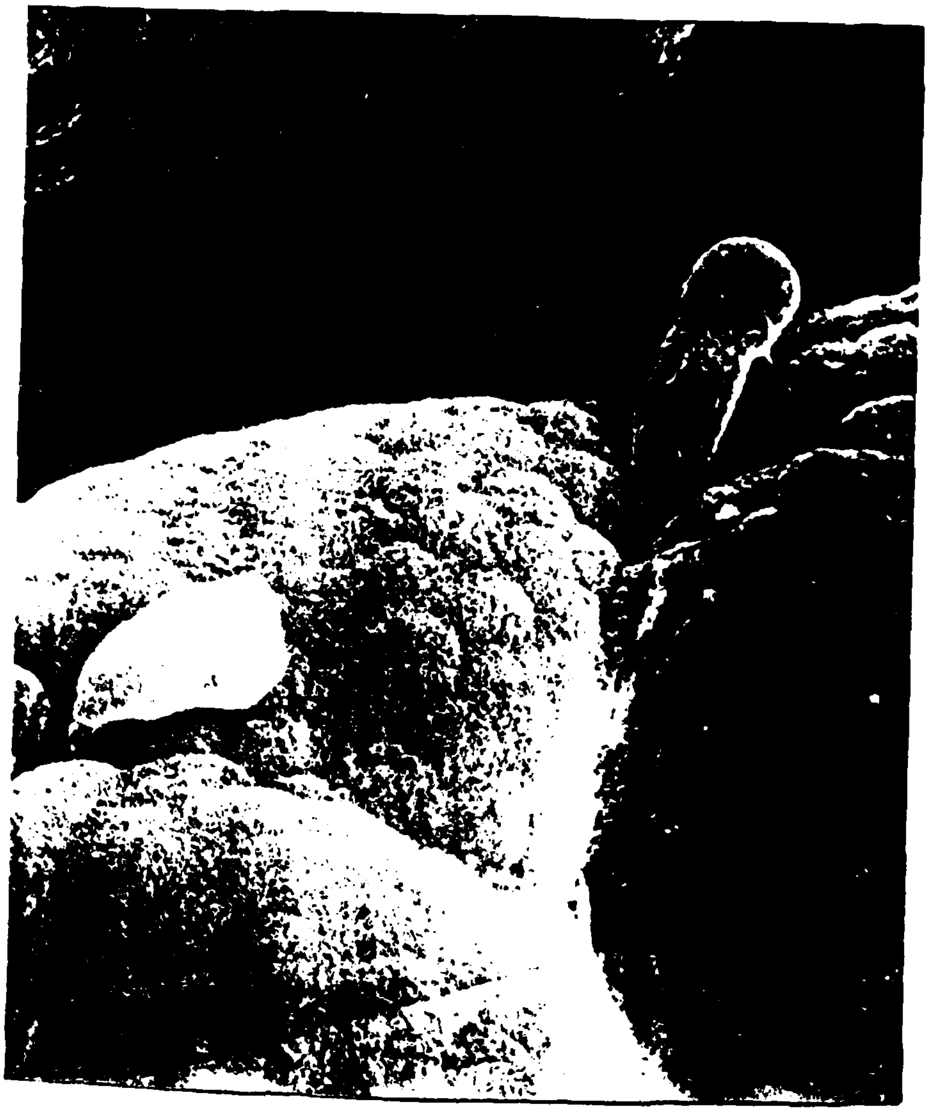

Fig. 1 SEM of proximal gastric mucosa (2400 X) demonstrating 2 cells in the process of exocytosis.

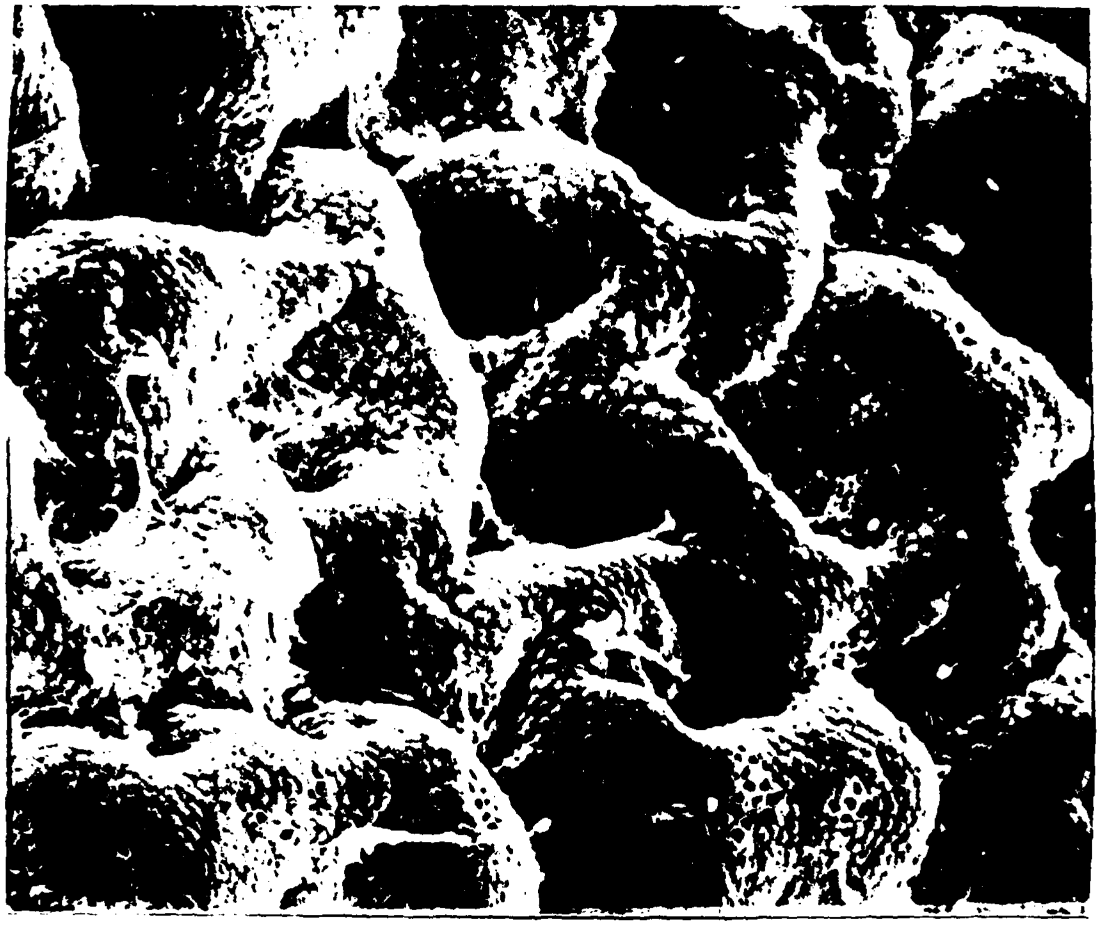

Fig. 2 SEM of proximal gastric mucosa (350 X) exposed to NTS + 5 TC. Exocytosis is present but otherwise the surface epithelium is intact. Note that the intrafoveolar ridges are carpeted with intact SECs.

Fig. 3 SEM of proximal gastric mucosa (330 X) treated with NTS + 5 C. The appearance of the mucosa is essentially the same as that in fig. 2.

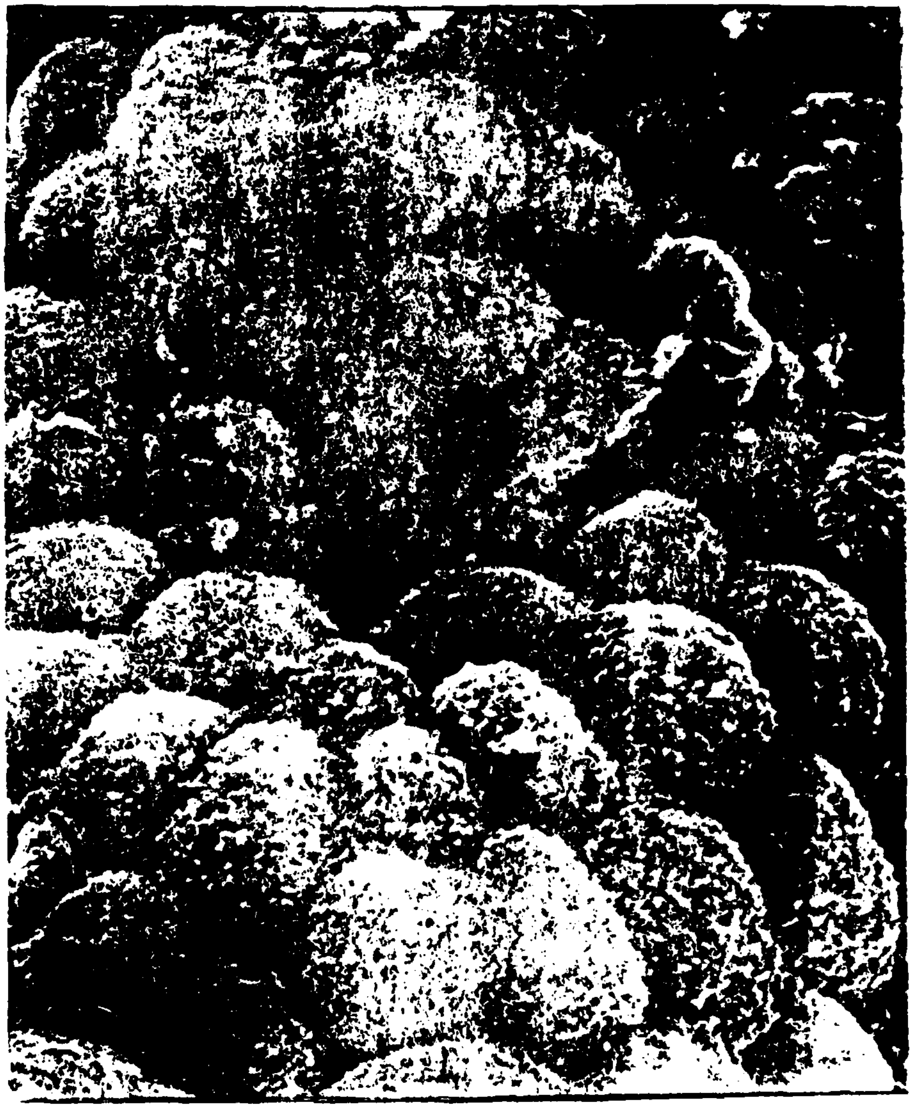

Fig. 4 SEM of proximal gastric mucosa (4500 X) demonstrating the space left by a cell which has undergone exocytosis.

Fig. 5 SEM of proximal gastric mucosa (8300 X) demonstrating expulsion of an apical mucus plug from an SEC.

Fig. 6 SEM of an perpendicular cut through the proximal gastric mucosa (6400 X) demonstrating 4 SECs which have undergone apical mucus expulsion. The cells remain attached to the basement membrane and to adjacent SECs. Mucus granules are apparent in the upper portion of each.

Fig. 7 SEM of proximal gastric mucosa (4500 X) showing 4 SECs which have expelled their apical mucus plugs. Adjacent cells have released lesser amounts of mucus, accounting for the irregularity of their apical membranes.

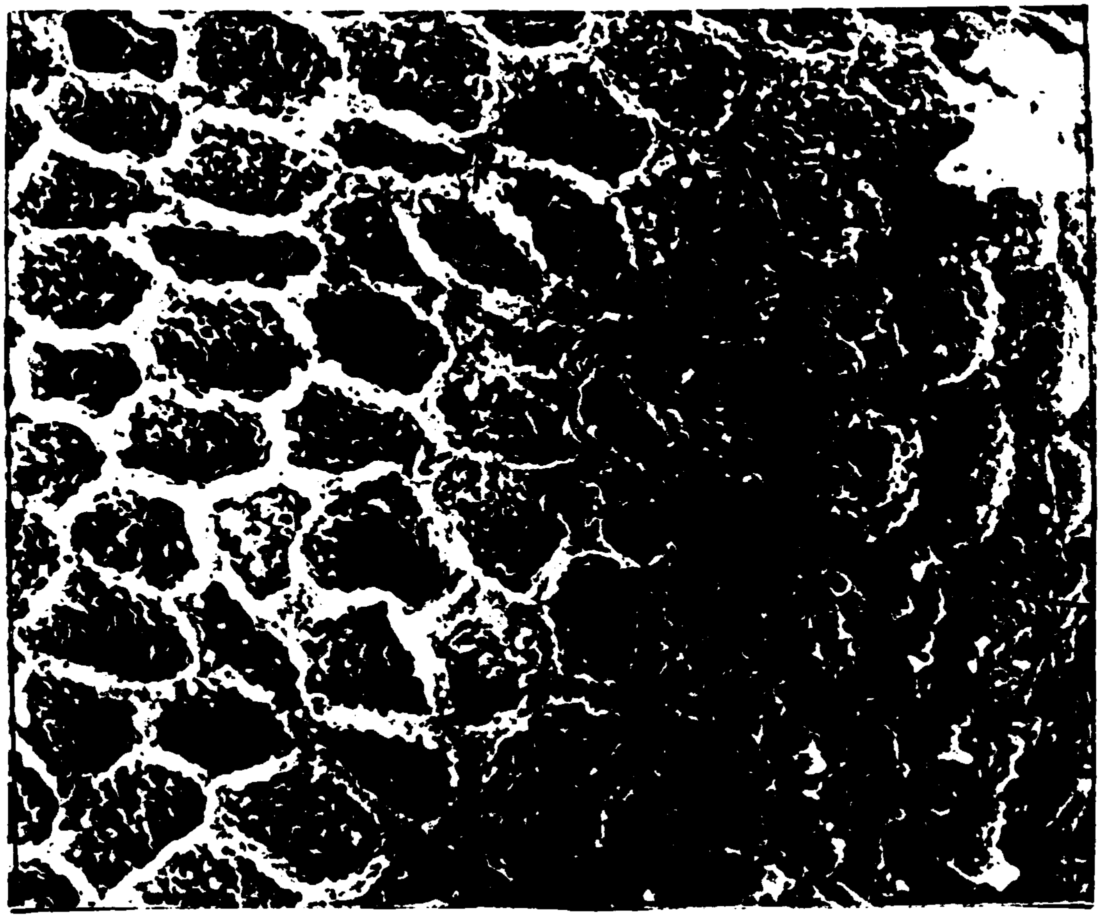

Fig. 8 SEM of proximal gastric mucosa (3300 X) demonstrating the typical honey-comb appearance of the mucus membrane when many cells have expelled their apical mucus plugs. Note the continued attachment of the cells to each other by the lateral membranes.

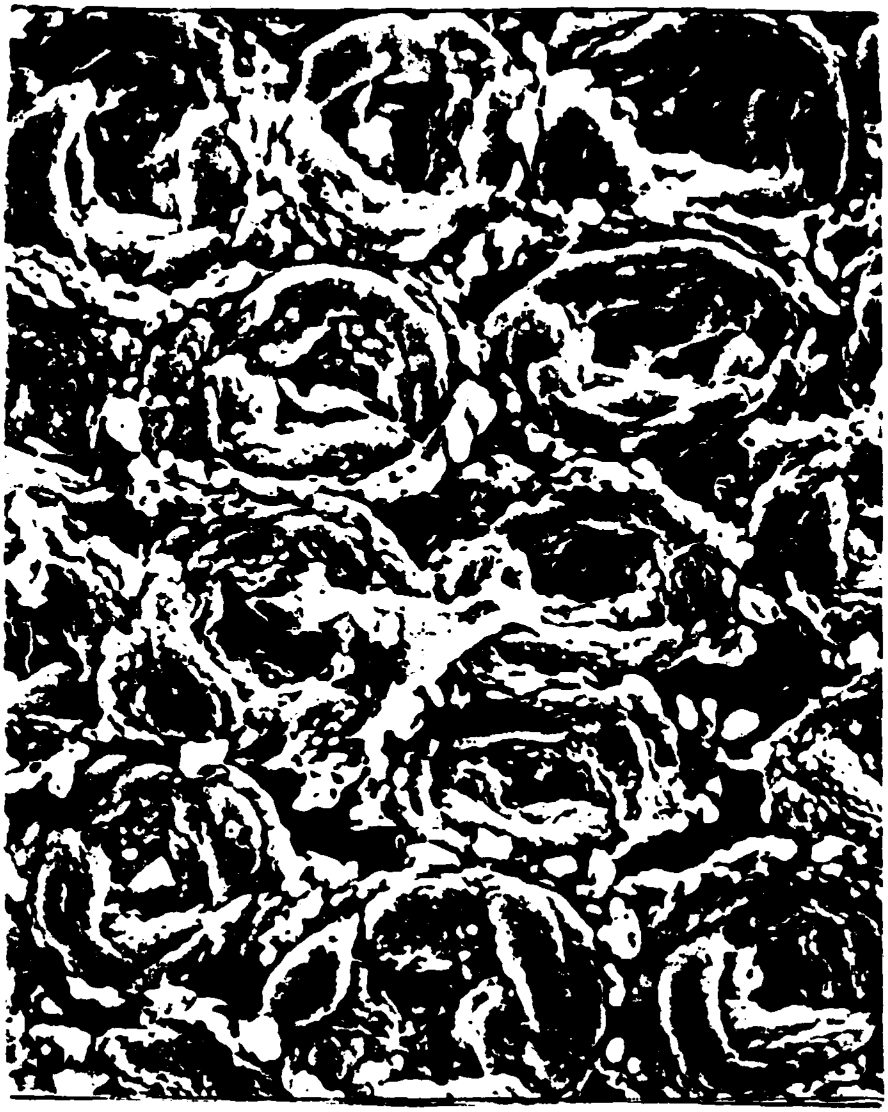

Fig. 9 SEM of proximal gastric mucosa (400 X) exposed to ATS following pretreatment with 5 C at pH 7. Surface epithelial cells in the region of the gastric pits are intact but the intrafoveolar ridges contain numerous non-viable SECs and, in some areas, the underlying lamina propera is completely exposed.

Fig. 10 SEM of proximal gastric mucosa (1700 X) showing a higher powered view of the same phenomenon as in fig. 9. Entire sheets of SECs have been or are in the process of being desquamated. Numerous non-viable cells are apparent in the intrafoveolar region, as evidenced by apical umbilication, cellular swelling, and non-attachment to either the basement membrane or to adjacent cells.

Fig. 11 SEM of proximal gastric mucosa (1100 X) demonstrates complete SEC desquamation around the entire circumference of a gastric foveolus. The process actually extends into the gastric pit for a short distance.

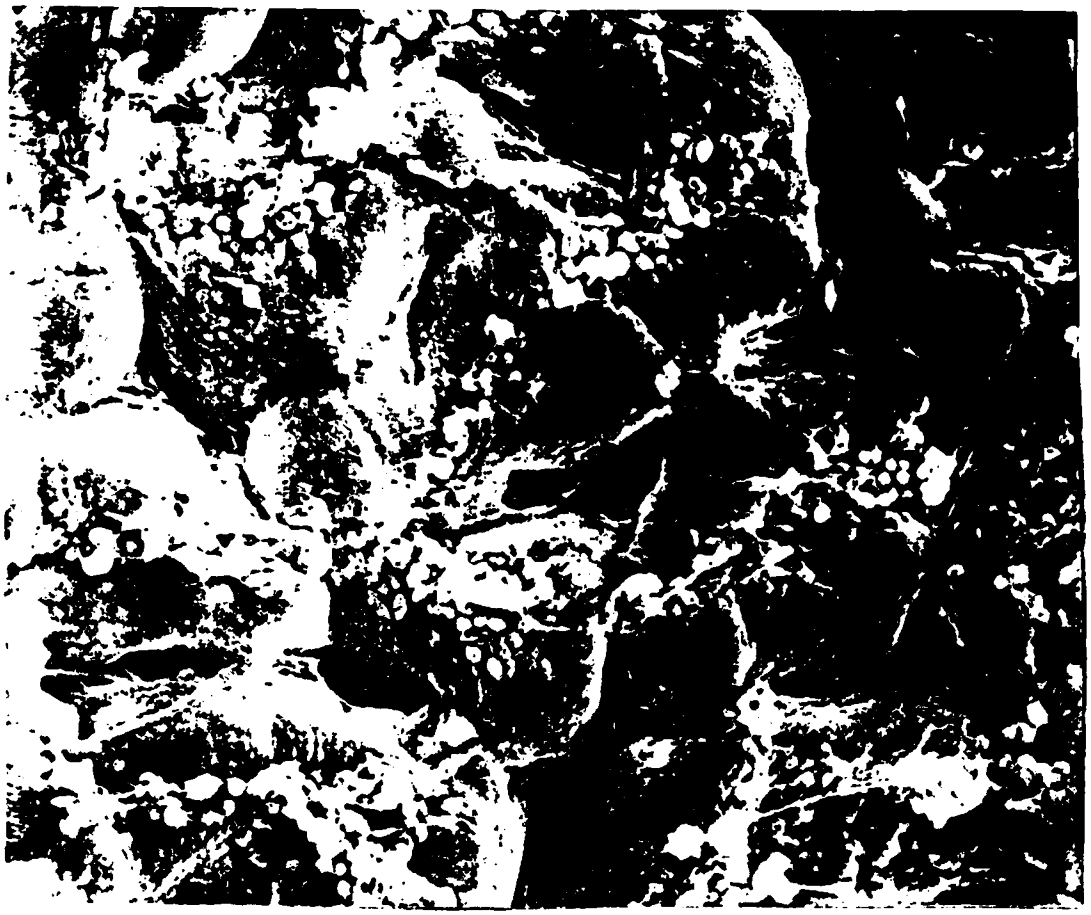

Fig. 12 SEM of proximal gastric mucosa (400 X) exposed to ATS after pretreatment with NTS + 2.5 mM C. The same process as illustrated in fig. 9 - 11 is present but to a lesser degree.

Fig. 13 SEM of proximal of gastric mucosa (2000 X) showing an intra-foveolar ridge in mucosa exposed to acid following pretreatment with 5 mM C at pH 7. As in fig. 10, numerous non-viable cells are present and the lamina propria is exposed at the top of the electron micrograph.

Fig. 14 SEM of proximal gastric mucosa (1800 X) showing an intra-foveolar ridge in mucosa exposed to acid following pretreatment with 2.5 mM C at pH 7. The central portion of the ridge contains non-viable SECs in the process of desquamation but the total area involved is markedly less than in fig. 13 and the lamina propria is not denuded. A rouloux of RBCs can be seen in the mouth of the gastric pit to the right.